PICRIC AND CHROMIC ACID FOR THE RAPID PREPARATION OF TISSUES FOR CLASSES IN HISTOLOGY.

SIMON HENRY GAGE, Ithaca, New York.

The standard methods of hardening tissues and preparing them for sectioning require so great an expenditure of time that it is practically impossible for students in college and carrying on other university work to perform all the processes and to make any satisfactory progress in the limited time devoted to histology. Believing firmly that unless a student learns to take every individual step himself in histology, as in all other branches of sound learning, the great object is unattained, I have been experimenting for the last few years in the laboratory hoping to so shorten and modify existing methods that every step may be taken by the student himself without too great an expenditure of time. The following are the results, and they are given not because they are the best possible methods that might be used if unlimited time were at the disposal of the student, but as methods that give excellent results in a very short time.

Picric-Alcohol Method.

The hardening and fixing solution consists of equal parts of 95 per cent. alcohol and water, and to this is added 1–5th per cent. of picric acid crystals, thus:

95 per cent. ethyl alcohol - - - -	1250 cc.
Water - - - - - - - - - - -	1250 cc.
Picric acid crystals - - - - - -	5 grams.

Or for a small amount:

95 per cent. ethyl alcohol - - - -	250 cc.
Water - - - - - - - - - - -	250 cc.
Picric acid crystals - - - - - -	1 gram.

The tissue is cut into pieces of moderate size and placed in a preserving jar containing about 25 to 50 times as much of the preservative as there is tissue. It is well also to suspend the tissue or support it on absorbent cotton, or to stir the tissue around occasionally. The tissue should be left in the picric alcohol about 24 hours. If the piece is small, 12 hours will do, and an immersion of 2 to 3 days seems to do no harm. After one day the tissue is placed for 24 hours in 67 to 70 per cent. alcohol, and then for one day or longer in alcohol of from 75 to 82 per cent. It may be left indefinitely in this. Finally, just before imbedding, the tissue is dehydrated one day only in 95 per cent. or stronger alcohol. It may then be infiltrated with paraffin or collodion in the usual manner, the whole time required being 7 days, at the longest, to harden, infiltrate, and imbed a tissue ready for sectioning.

The picric-alcohol method has given excellent results for all tissues except peripheral nerves. It is especially to be recommended for organs or parts possessing ciliated epithelium. The sections may be stained with almost any color, but hæmatoxylin and carmine are especially to be commended. It is further recommended that if too much of the yellow color of the picric acid has been removed in the various processes the final dehydration be performed, in part at least, with 95 per cent. or stronger alcohol containing 1–10th per cent. of picric acid, thus:

95 per cent. alcohol - - - -	100 cc.
Picric acid crystals - - - -	1–10th gram.

The double stain of hæmatoxylin and picric acid gives very sharply defined appearances, the hæmatoxylin staining the nucleus and the picric acid the cell-body and also the ground substance somewhat.

If ammonia carmine is used as a stain, more sharply differentiated appearances are obtained by dehydrating with the following:

95 per cent. alcohol - - - - -	100 cc.
Glacial acetic acid - - - - -	1 cc.
Picric acid crystals - - - - -	$\frac{1}{10}$th gram.

Nothing has been found more satisfactory for a clearing medium than a mixture of carbolic acid crystals, 2 volumes, and turpentine, 3 volumes, thus:

Carbolic acid crystals (melted) - - -	40 cc.
Turpentine (oleum terebinthinæ) - -	60 cc.

And for a mounting medium Canada balsam, dissolved to the consistency of thick syrup in xylol or cedar-wood oil, has given excellent results.

Flemming's Chrome-Acetic Acid Method.

This has proved satisfactory for the rapid fixing of peripheral nerves and for stratified epithelia. For the stomach and intestines it has not proved so satisfactory as the picric alcohol—chromic acid, ¼th per cent; glacial acetic acid, $\frac{1}{10}$th per cent. in water, thus:

Chromic acid crystals - - - - -	6 grams.
Glacial acetic acid - - - - - -	$2\frac{4}{10}$ths cc.
Water - - - - - - - - - - -	2400 cc.

The tissue is cut into pieces of moderate size and placed in 50 to 75 times its volume of the fixing agent for 12 to 24 hours. It is then washed two hours or more in water and left about 12 hours in 50 per cent. alcohol, then placed indefinitely in 75 to 82 per cent. alcohol. It may be dehydrated, infiltrated, and imbedded as described for the picric-alcohol method.

Hæmatoxylin is, on the whole, the most satisfactory stain, but the staining is not so satisfactory as after the use of picric alcohol. The staining may be hastened in this case, as in all others where it is desirable, by heating the staining agent.*

* If the picric alcohol solution, as given above, is diluted with an equal volume of water it makes a most excellent dissociating medium for almost all the tissues. It is especially good for epithelia and for smooth and striated muscle. The striation in the striated muscle is exceedingly clear and the longitudinal fibrillation of the smooth muscle is easy to demonstrate.

For a rapid and generally applicable method for hardening tissues see also the bichloride of mercury method described by Mr. Hopkins in his article on the structure of the stomach of Amia, described later in this volume.